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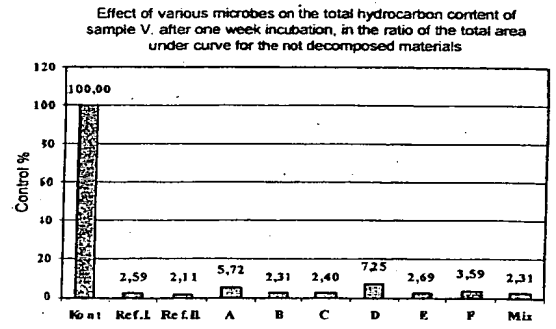
(54) Title: METHOD FOR THE TREATMENT AND PREVENTION OF ASPHALTENE-PARAFFIN-VAX PRECIPITATES IN OIL-WELLS, WELLHEADS AND PIPELINES BY THE USE OF BIOCOLLOID SUSPENSIONS



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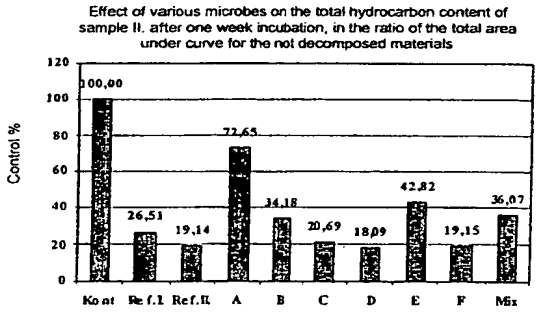

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(57) Abstract: The present invention relates to a method for the prevention, reduction and/or removal of asphaltene-paraffin-vax precipitates on surfaces in contact with crude oil within oil-wells from bottom hole to well head, in flow lines and pipelines by the use of biocolloid suspensions. In particular, the method comprises adding together (a) microorganism(s) capable of reacting with, solubilizing of and/or resistant to the components of crude oil, (b) organic or inorganic additives, or (c) a mixture thereof into the pipes, and the so formed suspension containing the microorganisms is allowed to act for a desired time. The said suspension can be used for the decomposition, washing-off and removal of solid hydrocarbons and mixtures of long chain hydrocarbons, as well as for the prevention of formation thereof.

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Method for the treatment and prevention of asphaltene-paraffin-vax precipitates in oil-wells, wellheads and pipelines by the use of biocolloid suspensions

The present invention relates to a method for the prevention, reduction and/or removal of asphaltene-paraffin-vax precipitates on surfaces in contact with crude oil within oil-wells from bottom hole to wellhead, in flow lines and pipelines by the use of biocolloid suspensions. In particular, the method comprises adding together (a) microorganism(s) capable of reacting with, solubilizing of and/or resistant to the components of crude oil, (b) organic or inorganic additives, or (c) a mixture thereof into the pipes, and the so formed suspension containing the microorganisms is allowed to act for a desired time. The said suspension can be used for the decomposition, washing-off and removal of solid hydrocarbons and mixtures of long chain hydrocarbons, as well as for the prevention of formation thereof.

The term "asphaltene-paraffin-vax precipitate" means in general a mixture of natural crystalline crude-oil paraffins, solid asphaltenes and/or solid hydrocarbon resins having high degree of dispersion and organic content, which precipitates from the crude oil and sticks to the walls of oil producing equipment to decrease the cross section thereof. The precipitation takes place upon the decrease of the temperature and pressure, as well as upon the change in the composition of the well-stream.

The term "film carrying bacteria" or "bacterium-carrying film" is defined herein as a continuous film in the physicochemical sense, which binds well to metal surfaces soiled with hydrocarbons, and at the same time provides for the living conditions for the bacteria used. Preferably, the viable bacterial layer forms a part of the film.

"Tenside" means any surfactant.

A "material increasing viscosity" defined herein as an organic or inorganic compound that changes the viscosity and rheological properties of a liquid having Newtonian rheological properties when admixed therewith, producing a colloid system having soft, plastic rheological properties.

The term "microorganism" is meant herein as a living organism, either of mono or multicellular structure or without and cellular structure, preferebly monocellular organisms, which belong to the scope of microbiology. "Microorganisms" are preferebly algae, in particular blue algae; bacteria and fungi.

A "microorganism strain" is a pure culture of microorganisms started from a single cell, preferebly a culture of a given species maintained or maintainable by regular subculturing.

One of the main problems of crude oil production is the removal of the mixture of long chain solid hydrocarbons, paraffins, vaxes and asphaltenes deposited from the crude oil onto the walls of production devices. A wide variety of physical and chemical methods are used these days in the field of crude oil production and transport for decreasing and removal of asphaltene-paraffin-vax precipitates. It is known from the art and industrial experience that the efficiency of every significant demulsification technique depends on a series of physico-chemical and technological parameters, which determine the intensity, location and characteristics of the asphaltene-paraffin-vax precipitates.

In practice, scrapers are widely used for cleaning the walls of production pipes of oil-wells from asphaltene-paraffin-vax precipitates. These are lowered down into the well on a steel cable with a reel using human power or machinery at predetermined time intervals, generally every few days, to a depth of 300 to 400 m. This method has several disadvantages:

(i) the scraper with the solid hydrocarbon stuck to it narrows the cross-section of the pipe, therefore acts as a local resistance or piston, which is not desirable under certain circumstances;

(ii) due to the breakage of the steel cable, the scraper often remains in the production pipe of the oil-well, the rescue of which causes significant production losses and extra cost;

(iii) special devices, personnel and experience is necessary for the movement of the scraper within the production pipe;

(iv) the application of scraper only solves the cleaning of tubing, but, at the same time, the deposition of asphaltene-paraffin-vax precipitates continues at the wellhead and near the well, thereby decreasing the flow cross-section.

Other physical methods for the cleaning of flow line and transfer pipelines of oil-wells include the use of different mechanical devices made of various materials, such as rubber balls, polyurethane pig, pipeline-scraper, etc. This method has several disadvantages:

(i) special loading and receiving chambers and locking system is necessary for the insertion and removal of the mechanical cleaning devices at the receiving stations;

(ii) the cleaning devices are often stuck in the pipelines, consequently causing production loss and their removal is very expensive;

(iii) these devices are sensitive, therefore their replacement is essential after a certain time.

Physical methods used in practice also include thermal procedures, which involve the periodical compression of hot crude oil, light hydrocarbon condensate, dry water vapor into the production pipes, annulus of the wells, flow line and/or transfer pipelines. This method has several disadvantages:

(i) the transportation and heating of materials necessary for the treatment is difficult to carry out, especially during winter, it requires machinery and energy, and also can pose danger of fires and accidents.

(ii) the production or transport must be stopped in certain cases to carry out the treatments.

Compared to the physical methods, the chemical methods are more universal. They can be performed by using a wide variety of chemical compounds. Different methods are characterized by the specific effects of the reagents. These include the followings:

(i) use of solvents in such an amount that is sufficient for the prevention of crystallization of paraffin under the given technological conditions. gasoline, heavy ends and other hydrocarbon-based solvents can be used as solvent. When the amount of vax and asphaltene components is high in the precipitate, large aromatic hydrocarbons are used.

(ii) use of materials for decreasing the freezing-point of the oil (dispersants) which, by their absorption on the paraffin crystals, helps to maintain the crystals in a finely dispersed form within the liquid. At the end, the intensity of the deposition of asphaltene-paraffin-vax organic complexes onto the walls of pipes and devices decreases.

(iii) use of surfactants to form a coat that prevents the attachment of asphaltene-paraffin-vax precipitates onto the surfaces of pores, pipes and devices;

(iv) use of inhibitors, which intensify the crystallization of paraffin within the crude oil, thereby increasing the number of small crystals, and preventing (inhibiting) the further enlargement and clumping. The small paraffin crystals and the vaxes, asphaltenes attached thereto are carried further by the flow, thereby the probability of their deposition is decreases;

(v) use of complexing chemical compounds, which have triple effect based on their composition: partial dissolution of asphaltene-paraffin-vax mass, dispersion of paraffin crystals and wetting of the surface of the devices by the activity of surfactant in the reagent.

Besides the several advantages of the chemical methods, the following disadvantages are present:

(i) most of the reagents are prepared in chemical factories far from the site of application and by the use of complicated processes, therefore the price and transportation means significant extra cost;

(ii) in case of diluents, amounts necessary for the treatment (3 to 35 m^3) compensates the relatively low price, and the treatment itself must be repeated at every one to two months;

(iii) in order to achieve long-lasting effects, in most of the cases reagents must be fed continuously, which requires construction of specific well equipments, injection pumps. There are oilfields where the older technology does not enable continuous feed, and the periodical treatments are not sufficient to achieve the desired results.

In addition to the above listed methods, microbial procedures and microorganisms are used to remove and/or inhibit asphaltene-paraffin-vax precipitates [Teh Fu Yen, Microbial Enhanced Oil recovery: Principle and Practice, CRC Press Inc. Boca Raton, Florida (1990].

This method is based on the properties of specific crude oil degrading bacteria to be able beeing adsorbed onto the surface of solid hydrocarbons, amongst them on the surface of asphaltene-paraffin-vax precipitates, which serve as nutrient source for the microorganisms [N. A. Lebedev et al., Prospects for microbiological technologies development in XXI century, Neftjanoje Hozjajtvo, 11/200]. Direct contact between the cells and solid substrate is not only advantageous, but essential for the hydrocarbon-degrading microorganisms. Adsorption of microorganisms changes the surface properties of the adsorbent and adsorbate, i.e. the surface of the asphaltene-paraffin-vax precipitates and adsorbed cells. The interaction between the cell surface and solid phase is effected by the protein complexes, lipids, polysaccharides and glycoproteins of the microbial cell wall. In addition, cells within the liquid phase, and especially the adsorbed ones, produce and secrete alcohols, fatty acids, biopolymers and exoenzymes into the environment during

their life cycle [Broshures of Micro-Bac International, Inc., USA (1990)]. Due to the adsorption, the dispersion of exoferments and organic hydrolizates is decreased, which are concentrated in close proximity to the surface of adsorbed cells. Consequently, a hydrophilic layer is formed on the hydrophobic surface of asphaltene-paraffin-vax precipitates, which is consisted of cells and metabolites.

Thus, based on its effects on the asphaltene-paraffin-vax precipitates, the microorganism suspension can be characterized as an adhesion, wetting, hydrophilizing, coating agent, as well as a natural inhibitor, due to its wash-off effect.

The action of adsorption inhibitors is based on the hydrophilization of the inner wall of the production pipe and pipelines, by the formation of a high molecular weight polymeric adsorption layer. Such adsorption layer is formed by the bacterial cells themselves. The bacterial cell wall is a complex system of a multitude of compounds, in which the effect of hydrophobic and hydrophilic molecules is combined. The high molecular weight, carbohydrate type compounds produced during the life cycle of bacteria, and enrichment thereof in the proximity of cells, also facilitates the polarization of surfaces. Production and excretion of lipid metebolites into the system treated decreases the interfacial tension between water and oil, thereby these compounds act as surfactants.

The production of natural inhibitors, differing from the chemical inhibitors which are washed off and diluted from the wall of the production pipe in relatively short time and require repeated treatments for the desired effect, depends on the conditions within the oil well and on the concentration and type of microorganisms, and it is a self-sustaining, renewing process. Another significant difference is that adsorption of microorganisms occurs not only on the walls of production and other pipes but on the surface of asphaltene-paraffin-vax precipitates, thereby there is no need for thorough cleaning to expose the metal surfaces prior to the treatment with the bacterial suspension.

Inhibition of asphaltene-paraffin-vax precipitates can also take place through their complete or partial metabolism. In this case the C-C linkages of the paraffin molecules brake up, and this process continues until the solid molecule is converted into a mobile liquid phase [I. Lazar, A. et al., The use of naturally occurring selectivity isolated bacteria for inhibiting paraffin deposition, JPSE, ELSEVIER 22, 161-169 (1999)].

The bacterial strains used in the microbial compositions used for the treatment of asphaltene-paraffin-vax precipitates are selected from the group consisting the following

bacteria: *Rhodococcus*, *Candida*, *Pseudomonas*, *Acinetobacter*, *Arthrobacter*, and other hydrocarbon-degrading microorganisms. If the conditions are suitable, these bacteria are capable of being adsorbed onto the surface of solid hydrocarbons and onto the inner walls of pipes, as well as able to break up enzymatically the C-C linkages of the precipitates.

Thus, it is very important to improve the methods having different efficacy for the removal of asphaltene-paraffin-vax precipitates from oil-producing wells to improve the efficiency of oil production and to decrease costs.

It was surprisingly found that by a specific combination of chemical and microbiological methods a significant improvement can be achieved in the removal and prevention of asphaltene-paraffin-vax precipitates formed in the oil producing wells, flow lines and pipelines.

Summary of the invention

In the method according to the invention, microorganisms selected in an appropriate way are used, and a "film carrying bacteria" is formed in the treated pipes by the use of a combination of appropriate additives. This film provides the living and operating conditions for the microbes. In addition to this, the additives of the microorganisms used for the inhibition of the formation of asphaltene-paraffin-vax precipitates are supplemented with tensides and materials increasing viscosity, which, in part or on the whole, are biodegradable. These are advantageous from a technological standpoint, and on the other hand, provide a retard carbon source for the microbes, therefore their use can provide the approach and attachment of microbes to the inner metal surfaces of the pipes for a longer period of time, and can provide for the living conditions thereof. It was especially surprising to find that the combination of the additives according to the invention enables the long-standing survival of a self-sustaining bacterial layer on the wall of the pipe. This, in contrast to the state of the art solutions, enables the continuous cleansing of the surfaces in contact with crude oil. Without restricting the scope of the invention by theory, the survival of the bacterial layer on the surfaces in contact with crude oil might be due to the combined effects according to the invention, of the additives used. In this respect, it is especially noteworthy that by the addition of materials increasing viscosity to the film carrying bacteria, the inherently viscous precipitates can be broken up, therefore decrease of their viscosity is achieved.

Therefore, the present invention relates to a biotechnological method, comprising the addition of selected microorganism or mixtures thereof in very high (10^6 to 10^{12} CFU per liter, preferably 10^7 to 10^{11} CFU per liter, more preferably 10^8 to 10^9 CFU per liter) amount in a fairly high (100 to 1000 liter per 100 meter pipe-length, preferably 500 liter per 100 meter pipe-length) volume with optimally balanced additives and carriers into the pipes to be treated in the form of gel suspension, and the said suspension is allowed to act for a desired time period.

Brief description of the figures

In figure 1, colonies of isolated bacteria can be seen streaked (inoculated) on a thin film of pollutant in three Petri-dishes. It can be observed that the pollutants are decomposed or converted surrounding the colonies. This can be seen as clearing or discoloration around the colonies. Whether we want to characterize the activity of decomposition, we can measure the width (diameter) of the cleared up (discolorated) band.

In figure 2 the ability of the acquired microorganism strains (phyla) to produce tensids is examined. With the hydrophilic – hydrophobic drop test one can observe the difference between spreading and non-wetting drops.

Figure 3 shows the effect of several microorganism strains – described using chromatography – on the hydrocarbon content of a paraffin sample (for V. see figure 3a , for II. see figure 3b) after one week of incubation. In the bar chart the ratio (expressed in percentage) the area below the curve characteristic of the undecomposed sample can be seen in the ratio of the area below the curve of the whole undecomposed mass. The marks on the horizontal axis mean the following microorganism strains.

Ref I	Hegrem*	
Ref II	Hegboost*	
A	MOL-2	NCAIM (P) B 1304
B	MOL-32	NCAIM (P) B 1305
C	MOL-51	NCAIM (P) B 1306
D	MOL-66	NCAIM (P) B 1307
E	MOL-107	NCAIM (P) B 1308
F	MOL-113	A *Pseudomonas sp.* strain isolated by the inventors

* commercialized by Oil Cleaning Bio-Products Ltd. P.O.Box 46, Royston, Hertfordshire SG8 9PD U.K., see also the product descriptions and the home page: www.ocbp.co.uk.

Figures 4a and 4b show the flow characteristics of crude oil samples form the oil-wells Alg-556 and Do-55, before the treatments of asphaltene-paraffin-vax precipitates, and after 3 treatments.

Figures 5a and 5b show the microscopy of the asphaltene-paraffin-vax precipitated from the same crude oil samples before (5a/1 and 5b/1) and after (5a/2 és 5b/2) the treatment.

Figures 6a and 6b show the output and composition of the same wells from oil samples before the treatment, and after three treatments.

Detailed description

The present invention relates to a method for the removal of asphaltene-paraffin-vax precipitates and prevention of formation thereof on surfaces in contact with crude oil comprising

a) adding tensides, materials for increasing viscosity, and microorganism(s) capable of breaking down crude oil components or derivatives and producing at least one type of tenside, to the surface, optionally together with additives required for the reproduction of said microorganisms;

b) providing an appropriate temperature for the microorganisms after the addition of the materials in step a);

c) allowing the microorganisms to reproduce and act for a predetermined period of time on the surface;

d) checking the results of the treatment; and

e) optionally repeating steps (a) to (d) at least once more, preferably at least three more times.

According to the invention, microorganism(s) capable of decomposition, washing-off and inhibition and removal of the formation of mixtures of long chain solid hydrocarbons forming the said asphaltene-paraffin-vax precipitates are used, which are optionally resistant to said mixtures. According to the present method, microbes capable of destroying and/or inhibiting asphaltene-paraffin-vax precipitates can be isolated in advance from production wells, pipelines or crude oil containers. On the other hand, commercially available microorganism, which are capable of decomposing paraffines, vaxes and asphaltenes and/or producing tensides, as well as genetically modified forms thereof can be used. The microbes used, with respect of their thermal needs, can be normal-intermediate (mesophil) or favoring a temperature higher than usual (thermophil). With respect of their

oxygen needs, anaerobic and facultative anaerobic microorganisms can be used. Furthermore, the microbes used for the present invention have to, in part, destroy and/or solubilize the materials of the precipitates, in a way that they produce certain materials (enzymes and/or tensides) *in situ*, which in turn capable of modifying the colloidal structure of the precipitates, and therefore breaking up the precipitates and/or preventing the attachment of precipitates to the wall of the pipe. In certain cases there can be a demand that the microorganisms used for oil degradation be apathogenic, in other words they shouldn't cause neither plant, nor animal, nor human diseases. In other cases even microorganisms capable of causing diseases can be used, if later they die, or if they have no effect on humans, thus can be used as a pesticide or herbicide at the same time. The microorganisms suitable for carrying out the technology of the invention, as mentioned hereinbefore, are commercially available, or alternatively can be some of the *Pseudomonas sp., Xanthomonas sp.* strains isolated by the present inventors.

Such microorganism strains suitable for the present invention can be prepared by the use of standard selection techniques known to the person skilled in the art, by culturing on appropriate selection medium, and selecting the strains showing the desired growth properties. Preferably, known microorganism(s), capable of decomposition, washing-off and inhibition and removal of the formation of mixtures of hydrocarbons (optionally resistant to said mixtures) can be used as starting material for the selection. Alternatively, bacteria can be cultured from oil production wells, from crude oils and oil containers, possibly from soils contaminated with oil, and then these bacteria can be further selected based on their effects on large molecular weight asphaltene-paraffin-vax precipitates. Such selection method is disclosed in the P0203394 Hungarian patent application, the disclosure of which is incorporated herein be reference.

In a preferred embodiment, the microorganism suitable for the method according to the invention can be selected as follows:

i) applying a film comprising mineral oil component(s) or derivative(s) to a minimal medium lacking carbon source,

ii) inoculating this medium with a sample comprising a mixture of microorganisms said sample being obtained from an oil pollution, and incubating the medium after inoculation at least till detectable microorganism colonies are formed; if the formation of colonies does not occur within an arbitrarily defined time period step i) and present step ii) are repeated,

iii) decomposing activities of the microorganism from the colonies formed are tested at the surroundings of the colonies, and

iv) tenside producing abilities of the decomposing microorganisms obtained from the colonies are checked and a tenside producing microorganism is selected.

Preferably, the microorganism is a facultative anaerobic which is obtained in the above method by using minimal medium comprising materials facilitating anoxic respiration, preferably electron acceptors and/or oxigen sources – in particular one or more of the following: Ti-compounds, Mn-compounds, nitrite, nitrate, phosphate, pyrophosphate ions or their salts, and preferably the incubation is carried out at least partly under anaerobic conditions.

The decomposing activity preferably is assessed by assaying the pollutant concentration of samples taken from the close surround/immediate vicinity of the colonies and/or on the basis of the diameter of the decomposed area. As a decomposing activity e.g. paraffin decomposing activity can be assayed or an enzyme activity for decomposing typical mineral oil pollutions, preferably by sampling, solvent extraction then by gas chromatography.

In a preferred embodiment, tenside producing ability of the microorganisms from the colonies obtained can be studied by e.g. a hydrophobic-hydrophilic drop test.

If microorganisms are isolated from the environment, so called sterile "solid minimal cultural-media" or preferably "silicagel solid culture-media" is used (for example in Petri-dishes).

Whether we isolate microorganisms capable of aerobic and anoxic activity it is advised to use culture-media containing nitrogen, sulphur, phosphorous salts and agar-agar, preferably sterile silicagel solid culture-media.

It is important to administer the specific hydrophobic pollutant or other hydrophobic compounds (hydrocarbons, crude oil, or its components and their derivatives) to decompose, dissolved in some kind of solvent, for instance a certain volatile organic solvent (alcohol, acetone, ether), preferably in pentane, hexane, or in methyl-benzene in the form of a thin film. Then the selected microorganisms from a fresh culture should be streaked onto this pollution layer, afterwards it should be incubated in the appropriate conditions for the strains (psychrophil, mesophil, thermophil, and aerobic, or anaerobic). After a certain time the microorganisms resistant to the pollutant and are able to

decompose it will form colonies usually consistent or showing characteristic morphology or pigmentation.

The microorganisms release enzymes into the area around the colonies, which are capable of decomposing the hydrophobic compounds such as hydrocarbons, and tensides are released also.

The enzyme production can be characterized by the width of the band (clearing up or discoloration) surrounding the colonies. This characterizes the intensity of the enzyme production mainly (figure 1). The produced enzyme activity can be determined by taking samples from the surrounding area of the colonies and we determine the composition of the pollutant by the means of gas chromatography. The microorganisms showing the highest enzyme activity are then selected.

The microorganisms producing tensides can be selected according to the hydrophilic-hydrophobic examination (for instance by water drops then by paraffin drops; see figure 2).

Depending on the conditions of the selection of the microorganisms we can acquire information concerning their essential conditions besides their activity of decomposition. Thus microorganisms used for bioremediation can be ones that prefer cold (psychrophilic), the ones that prefer medium temperature (mesophilic), or the ones that prefer temperature above normal (thermophilic).

Using the above-mentioned selection method, the present inventors isolated *Pseudomonas sp.* and *Xanthomonas sp.* microorganisms from oil polluted soils, the following of which were deposited on April 17, 2002 at the National Collection of Agricultural and Industrial Microorganisms (1118 Budapest, Somlói út 14-16) according to the Budapest Treaty:

MOL-number	Deposition number
MOL-2	NCAIM (P) B 1304
MOL-32	NCAIM (P) B 1305
MOL-51	NCAIM (P) B 1306
MOL-66	NCAIM (P) B 1307
MOL-107	NCAIM (P) B 1308

Microorganisms can be genetically enhanced, favorably carrying DNA fragment - of which the sequence is known – ligated into its' genomes as a marker.

In a preferred embodiment, the present invention further relates to a method wherein in step d) the results of the treatment are checked by confirming the presence of a film on the surface in contact with the crude oil which provides for the living conditions of the said microorganisms and contains the said microorganisms, and optionally steps a) to d) are

repeated by changing the parameters, preferably by changing the amount of the tenside or material capable increasing viscosity, or by varying the reproduction time of microorganisms.

The approach of the microorganisms to the inner metal surface of the pipe, the attachment and living conditions thereof is facilitated by tensides and macromolecules, preferably by biodegradable polymers, or by physiologically harmless solvents, detergents and/or tensides.

In preferred embodiments of the present invention, the materials used binds well to metal surfaces soiled with hydrocarbons. Due to this binding, a thin, continuous "film carrying bacteria" is formed on the contaminated metal surfaces from the additives used. This bacterium-carrying film provides the living conditions for the bacteria used. It is noted again that a viable, reproducing bacterial layer can be formed on the soiled surface of the pipe in the inventive method for the removal of contaminations. This bacterial layer localizes the effects described hereinbefore in the section on microbial treatments, and this focused effect allows an improved, more effective means for the removal of contaminations, or the prevention of formation thereof.

In further preferred embodiments, the said precipitates are removed from or prevented in the inner surfaces of pipes of oil-wells, flow line thereof, or in oil pipelines by the use of the method according to the invention.

The formation of the bacterial layer is facilitated in that the bacterium-carrying film covers the inner surface of the pipe in a prolonged, stable manner after its formation. This film attaches both to the free metal surfaces and to the surfaces contaminated with asphaltene-paraffin-vax precipitates, thereby providing for suitable cleansing of the inner surface of the pipe, as well as for preventing the formation of further precipitates.

The bacterium-carrying film of the invention has the advantageous property of stability against wash-off by the flowing mixture of hydrocarbons. This is essential for the long-standing maintenance of a viable bacterial population necessary for the cleanliness of the pipes in the treated oil wells, pipelines, etc., providing a self-sustaining cleansing mechanism. The method according to the invention may not eliminate completely the necessity for the physical cleaning techniques, however, it facilitates the performance thereof, and decreases the risk of the tear of the cleaning devices, and increases the time intervals between physical treatments.

In a preferred embodiment, the invention relates to a method wherein the microorganisms and additives are added into the pipes at the same time in the form of an aqueous suspension. The polymer materials forming the film are capable of maintaining most of the microorganisms on the surface of the pipe in the form of a suspension, not interfering with the reproduction of said microorganisms and with the production of metabolites. Thus, the bacterial suspension introduced by the treatment remains stably on the surface of the pipe, and due to its viability and metabolism, it continuously decomposes the contaminants consisting of asphaltene-paraffin-vax precipitates, which, at the same time, serve as nutrients for the metabolism of the said bacteria.

The invention also relates to a method wherein the suspension of microorganisms contains 10^6 to 10^{12} CFU/liter, preferably 10^7 to 10^{11} CFU/liter, more preferably 10^8 to 10^9 CFU/liter. According to this method, the volume of the suspension is 100 to 1000 liter/100 m pipe-length, preferably 300 to 800 liter/100 m pipe-length, more preferably 500 to 600 liter/100 m pipe-length.

In these preferred embodiments, the metabolites produced by the reproducing bacteria in the bacterium-carrying film formed from the starting materials used, and the reproducing bacteria themselves enter the flow of the hydrocarbons. These mobilized bacteria and metabolites, preferably having tenside and detergent activity, provide further means for the regeneration of the bacterium-carrying film, and for coating the free surfaces, or surfaces freed by the removal of the contaminating precipitates. This way the film will also be extended.

The film carrying the bacteria, together with the bacteria therein and attached to the wall of the pipes will hydrophylize the metal surface. This effect is facilitated by the additive components having surface activity, which are not interfering with the viability of the microorganisms. The preferred surfactants are selected from the group consisting of octil- or nonilphenoxy-polyethoxy ethanols (for example from the commercially available Triton™ series), polyoxyethylene sorbitan esters (Tween™ series) and the polyoxyethylene ethers or esters of the general formula (I):

$$HO(CH_2CH_2O)_n\text{-}A\text{-}R \qquad (I)$$

wherein n is an integer between 1 and 50, A is chemical bond or -C(O)- group, R is $C_{1\text{-}50}$ alkyl or phenyl-$C_{1\text{-}50}$ alkyl; or a combination of two or more of the above. In a preferred

embodiment the surfactant is selected from the group consisting of polyoxyethylene ethers or esters and mixtures thereof, preferably Tween 80.

The main assurance for the stability of the bacterium-carrying film is the use of materials increasing viscosity, to increase the relative viscosity of the additive mixture realtive to water. These additives are preferably macromolecular compounds. The use of said compounds allows and strengthens the attachment of the bacterium-carrying film and the bacteria therein to the inner surface of the pipe, and maintains the bacterium-carrying film on the surface. Non limiting examples of such macromolecular components according to the invention are Carbolpol, Supramil, xanthan, other water soluble macromolecules, such as starch, cellulose derivatives, and the like. According to an especially advantageous embodiment, the material increasing viscosity is xanthan.

The additives used to form the film carrying the bacteria may contain further components, such as dimethyl sulfoxide, cellosolv, methyl-cellosolv, and the like.

Additives to increase the viability, reproduction and/or activity of the bacteria can be preferably introduced into the system, which can be admixed to the film carrying the bacteria without decreasing its effectiveness. These additives can provide the nutrients for the microorganisms used, such as provide the necessary moisture, electron acceptors, macro and micro elements (materials to provide the necessary carbon, nitrogen, phosphorus, sulphur, etc.) in order to effectively inhibit the formation of asphaltene-paraffin-vax precipitates or prevent those in the pipes treated.

Furthermore, the activity of facultative anaerobic microbes can be enhanced by the use of electron acceptors to allow anoxic respiration, such as nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{3-}) or sulphate (SO_4^{2-}) and/or ferri salts. In addition, inorganic salts of other compounds, which also help anoxic respiration (NO_2, NO_3, PO_3, PO_4, P_2O_4, P_2O_7, ClO_4, BO_4, B_2O_7), or even organic compounds (dehydro-ascorbate, alpha keto-glutarate, acetic aldehyde, pyruvate, oxalic acetate, fumarate, humin acids, etc.) can be used [Chih-Jen Lu et al., The effect of electron acceptors on the nitrate utilization efficiency in groundwaters, in Hydrocarbon Bioremediation, pages 469-474, editors: R. E. Hinchee B. C. et al., Lewis Publisher, Boca Raton, FL].

Preferred additives to be used according to the invention are the following:

(i) carbon sources, preferably glucose, saccharose, molasses, glycerol, acetate, xanthan, etc.;

(ii) nitrogen sources, preferably peptone, essential amino acids, NH_4, NO_2, NO_3 or their salts;

(iii) phosphorous sources, preferably PO_4, P_2O_5, P_2O_7, or their salts;

(iv) sulphur sources, preferably sulphate, pirosulphate ions or their salts.

In the method according to the invention, the microorganism are allowed to reproduce and act for 1 to 15 days, preferably for 6 to 8 days, while the pipes are kept closed. In case when there is a possibility to modulate the temperature of the surface or pipeline, it should be set to a temperature which allows for the reproduction and activity of the microorganisms, preferably near to the optimal temperature thereof. The temperature used is typically between 20 to 98 °C, preferably between 40 to 80 °C, and in the case of moderately thermophyl bacteria, preferably between 50 to 70 °C, more preferably around 60°C.

In an alternative preferred embodiment, the method is carried out in a production oil well, and the temperature of the well is the temperature determined by the geological conditions. According to the method of the invention, the surface can be cleaned by mechanical means from the asphaltene-paraffin-vax precipitates, if desired. The microorganisms are allowed to act for several days in the treated pipes, in order to enable the formation of a hydrophilic layer having suitable adherence from the bacterial suspension, and further to enable the reproduction of the bacteria introduced by using up the starting nutrients. After the consumption of the nutrients, the production of metabolites will start, and the amount thereof may be sufficient to prevent the growth and adherence of the asphaltene-paraffin-vax particles formed in the flow of hydrocarbon mixture.

After carrying out the method of the invention, the results of the treatment are checked by pilot test and by mechanical cleaning test and/or by evaluating the physico-chemical properties, preferably the decrease of viscosity of an oil sample and/or evaluating the drop-size of the asphaltene-paraffin-vax precipitates in an oil-sample by microscopy.

The methods of the invention are preferably carried out by using the microorganism defined in the present specification.

The present invention further relates to the use of a microorganism capable of breaking down crude oil components or derivatives and producing at least one type of tenside for the removal and prevention of asphaltene-paraffin-vax by way of forming a film carrying bacteria on surfaces in contact with crude oil.

In a preferred embodiment, the invention relates to the use of a microorganism which is a strain belonging to the *Bacillus subtilis* species, the *Bacillus cereus* species, the *Pseudomonas* genus or the *Xanthomonas* genus, and preferably facultative anaerobic.

It is an especially preferred use wherein the microorganism is selected form the group consisting strains NCAIM (P) B 1304, NCAIM (P) B 1305, NCAIM (P) B 1306, NCAIM (P) B 1307 and NCAIM (P) B 1308 deposited on April 17, 2002 at NCAIM, or any strain derived therefrom, and preferably is a strain that is genetically modified, more preferably modified by the insertion of a DNA fragment with a known sequence as a marker.

In further embodiments, the invention relates to a kit for the removal or prevention of asphaltene-paraffin-vax precipitates on surfaces in contact with crude oil in pipelines, comprising a microorganism useful in the method of the invention, further comprising instructions to carry out the method of the invention.

The invention further relates to a kit comprising one or more of the microorganisms defined hereinbefore and additive(s) necessary for the reproduction thereof. In a preferred embodiment the kit further comprises a surfactant and/or a material for increasing viscosity.

The invention is further detailed in the following examples.

A. Selection of microorganism strains

1. Example – Cultures on minimal medium

Suspensions (1-20%) of samples containing materials of the said precipitates or a component thereof (mineral oil components, paraffins, asphaltenes, maltenes, etc., or derivatives of the mineral oil) are dispersed in physiological salt solution or even in any physiologically useable buffer with a pH 6.5-7.6 were made. Certain dilutions of such suspensions were administered onto the surface of agar-agar minimal culture media, and were incubated on 0 to 80 °C for a desired time, preferably for 12-72 hours. The isolated colonies were selected according to their activity of pollutant decomposition.

Agar-agar minimal culture-media (for 1000 g of distilled water):

0.1-3 g preferably 2.5 g Na_2HPO_4

0.1 to 3 g preferably 1.5 g KH_2PO_4

0.1 to 3 g preferably 0.5 g $(NH_4)_2SO_4$

0.01 to 3 g preferably 0.05 g $CaCl_2$

0.5 to 3 g preferably 2.0 g agar-agar

0.1 to 5 g preferably 1.5 g $NaNO_3$

It can be seen that the media contains ions promoting anoxic respiration (PO_4^{3-} and its protonated forms, SO_4^{2-}, NO_3^-) in other words it contains electron acceptors, which also allows the selection of aerobic and facultative aerobic microorganisms.

In certain cases the aforementioned media was supplemented with 1-50 mL, preferably 10 mL of the following solution (1000 mL):

0.1 to 0.5 g preferably 0.25 g H_3BO_4

0.1 to 1.0 g preferably 0.25 g CoCl

0.1 to 2.0 g preferably 0.25 g $CuCl_2$

0.05 to 2.0 g preferably 0.25 g $FeSO_4$

0.01 to 1.0 g preferably 0.025 g $MnCl_2$

0.01 to 1.0 g preferably 0.025 g $NaMoO_4$

0.01 to 1.0 g preferably 0.025 g $NiCl_2$

0.01 to 1.0 g preferably 0.025 g $TiCl_4$

The metal ions of other oxidative states (for example Ti, Mn, Mo ions) also promote anoxic respiration as redox systems.

2. Example: Silicagel culture media

The microflora of the polluted soil samples can be grown on so called "silicagel minimal culture-media" which is a version of Vinogradszkij type silicagel solid culture media which is supplemented with the compounds mentioned in example 1.

Thermophilic (50 to 80 °C) and extreme thermophilic (80 to 110 °C) microorganisms can be grown and selected on silicagel minimal culture-media.

3. Example: Examination of the activity of pollutant decomposition

The ability of decomposition of the microorganisms isolated from minimal culture-media can also be examined on such solid media. In this case we administer the hydrophobic pollutant (hydrocarbons, lipoids etc.), dissolved in some kind of solvent, for instance a certain volatile organic solvent (alcohol, acetone, ether), preferably in pentane, hexane, in the form of a thin film. Then the microorganisms to be examined should be streaked onto this pollution layer. (figure 1).

The colonies are incubated at the desired temperature with the given oxygen concentration, for a desired time, preferably for 12-96 hours, more suitably for 48 hours, then the method should be repeated preferably 2-3 times again with the cultures grown.

The controlled level of oxygen concentration allows us to perform our method in aerobic and anoxic conditions, thus we can isolate microorganisms which show activity in both aerobic and anoxic conditions. During the isolation of such facultative anaerobic microorganisms, part of the growth was done in anoxic conditions, and the media contained compounds that promote anoxic respiration.

When the microorganisms isolated in the aforementioned way were streaked onto the film of pollutant, in the area around the colonies clearing up and discoloration could be observed showing that the pollutant was either converted, or decomposed (figure 1).

Below we will introduce how we examined the effectiveness of decomposition, the ratio of pollutants decomposed after a certain time in the clearing (figure 3), thus the selected enzymes' activity was examined. Also we could examine the appearance of other compounds, specifically tensides, during the course of decomposition, which helped the process (figure 2). Of course a person skilled in the art can use other protocols in this case.

4. Example: Examination of the effect of Microorganisms

Activity of enzymes of oil decomposition

On the surface of 15 mL of minimal agar-agar or minimal silicagel culture-media in a sterile Petri-dish with a 10 cm diameter we administered a thin film of pollutant (crude oil products dissolved in 5% hexane or methyl-benzene solutions). Onto this film with a platinum loop we streaked the microorganisms isolated from a polluted environment (soil, ground water, etc), and grown in liquid media. Then they were incubated under the desired conditions (aerobic, or anaerobic), at the chosen temperature (15 to 20, 30 to 35 or 50 to 85 °C) for the desired time (24-240 hours), up until the microorganisms formed distinguishable colonies. In case we can observe a certain change in the hydrocarbon film (clearing, discoloration) we take samples from these zones, then extract it (hexane, methyl-benzene etc) with a solvent, then we examine the mineral oil product's quantity and its composition with the help of gas chromatography.

The effectiveness the production (also including the viability) of enzymes capable of decomposing oil can be characterized by the width of the zone of clearing. The activity of

the enzymes can be followed by the decrease of the quantity of hydrocarbon components of the rock oil products.

The activity of a few of the isolated microorganism strains is compared to other known strains (Table 1, 2). The letters in the table mean the following:

BO-1	Hegboost*	
RO-1	Hegrem*	
A	MOL-2	NCAIM (P) B 1304
B	MOL-32	NCAIM (P) B 1305
C	MOL-51	NCAIM (P) B 1306
D	MOL-66	NCAIM (P) B 1307
E	MOL-107	NCAIM (P) B 1308
F	MOL-113	A *Pseudomonas sp.* strain isolated by the inventors

* commercialized by Oil Cleaning Bio-Products Ltd. P.O.Box 46, Royston, Hertfordshire SG8 9PD U.K., see also the product descriptions and the home page: www.ocbp.co.uk.

Table 1. The effect of bacteria groups on paraffins with different melting points.

Sign of group	paraffin				
	DW 6266	DW 7580	DW 5456	DW 5658	DW 5052
BO-1 [e]	+ 6	+ 5	+ 6	+ 6-8	+ 4-7
RO-1 [e]	+ 6	+ 6	++ 4-11	+ 3-6	++ 5-12
A [t]	++++ 15-18	+ 5-8	+++ 10-15	++++ 11-19	+++ 11-16
B [t]	+++ 5-11	+ 5-6	+++ 10-15	++++ 13-20	+++ 10-16
C [t]	+++ 10-15	± 4	+++ 14-17	+++ 14-18	+++ 11-14
D [e(t)]	+ 5-7	+ 4-5	++++ 10-22	++++ 10-34	+ 4-7
E [e(t)]	+ 6-7	+++ 10-13	+++ 13-17	+++ 11-13	+++ 13-16
F [e(t)]	++ 9-12	++ 6-10	++ 7-12	++ 4-10	++ 7-12

t-effect of tensides

e-enzyme activity

activity

+ insignificant

++ partial

+++ satisfactory

++++ outstanding

number – the diameter of the decomposed area

Table 2. Different precipitated crude oil decomposition with bacteria groups at 37 °C after 96 hours.

Sign	hydrophobic[x]	asphaltene	maltene	5% asphaltene + Alg #571 oil
BO-1	+ 4-7	 9	+ 4-7	++ 6-12
RO-1	+ 4-10	 7	+ 4-8	++++ 15-18
A	+ 5	++++ 10-38	± 2	++++ 22-25
B	+ 4-8	++++ 14-20	+ 4-7	++++ 34-37
C	+ 4-6	++ 7-12	± 2-4	++++ 25-30
D	+ 3-6	+ 4	+ 4-8	++++ 30-35
E	++++ 22-25	+ 5-7	++++ 20-25	++++ 30-35
F	+ 4-5	+ 4-5	++++ 10-35	 20-35

t-effect of tensides

e-enzyme activity

activity

+ insignificant

++ partial

+++ satisfactory

++++ outstanding

number – the diameter of the decomposed area

While comparing figure 3 with the Tables one can see that the enzyme activity (measured with gas chromatography, i.e. GC) of our isolated strains was a match for the strains known up to date, the effectiveness of decomposition (characterized bye the

average width of the area cleared up as a band), considering the pollutant significantly exceeded that of the strains known up to date. With our technology microorganisms specifically selected can be produced and can be used specifically for a pollutant that they decompose the most effectively.

Detection of tensides produced with the help of hydrophilic-hydrophobic drops

We repeat the procedure mentioned in example 4 in case of oil decomposing enzymes, with the exception that in the clearing surrounding the colonies of the chosen microorganisms, under the desired conditions we administer a few drops of distilled water or melted paraffin onto the surface of the media. In the zone containing tensides the drop of distilled water spreads out, while in the area with no clearing up (hydrophobic) it forms a moveable bead like drop. The melted paraffin drop spreads out in a moveable manner in the area with tensides, while it sticks to the hydrophobic zone making its movement impossible (figure 2).

The surface critical angle of the drops is measurable, and can even be used to quantitatively describe the production of tensides if fixing other parameters (growth time, drop zone).

B. Treatment of oil wells

The method of the invention was tested on two oil wells: on the well designated Alg-556, located in the Algyő region (field of Alsópannon-13/b) and the one designated Do-55, from the fields of Kiskundorozsma. Prior to the treatment, tubings of the wells were deparaffinated by mechanical means, using the scraper technique. The cleaning of the Alg-556 well took 1 to 1.5 hours, at the end of which the lubricator need to slant for cleaning the scraper. Depth of the deparaffination was 350 too 400 m. During the cleaning of the well Do-55, the lubricator had to slant 2 to 3 times within 30 to 50 minutes time period in order to deparaffinate the production pipe in a depth of 300 to 350 m.

During the treatment of these wells, 1000–1200 liter of additives and a mixture of 10^{11} CFU/liter *Pseudomonas sp., Xanthomonas sp.* microorganisms [NCAIM (P) B 1307, NCAIM (P) B 1308] were used in the production wells of 1500 and 2500 m depth, respectively, repeated 3 times, as described in the specification.

The composition of the additives used is shown in Table 3.

Table 3. The composition of the treating fluid. Amounts shown are for 100 liter of treating fluid.

Component	Additive (g)	Preferred amount (g)	w/v %
Additive/nutrient			
Glucose	50-300	100	0,1
Saccharose	150-1200	620	0,62
Peptone	10-100	40	0,04
NH_4NO_3	20-400	140	0,14
Na_2HPO_4	30-500	125	0,125
Polar organic solvent			
DMSO	60-1000	250	0,25
Surfactant			
Tween 80	10-100	25	0,025
Material increasing viscosity			
Xanthan	20-400	100	0,1

Results

The data presented In figures 4 show that the method used resulted in significantly decreased viscosity.

According to figures 5, the structure of asphaltene-paraffin-vax precipitates within the flowing phase becomes loose, thereby not attached to the wall of the production pipe.

The data of figures 6 show that the production of the well Do-55 has increased, and the ratio of water and oil has also changed.

The method of the invention resulted in the improvement of the quantity and quality (flow characteristics) of the production within the treated wells, and, more importantly, the inhibition of asphaltene-paraffin-vax precipitates has been maintained continuously for 8 to 12 months. It can be seen, therefore, that the bacterium-carrying film formed in the treated pipes provides a self-reproducing protective coat.

The oil wells changed completely with respect to deparaffination. After the treatments, slant of lubricator and cleaning of scrapers was not necessary due to the insignificant amounts of paraffin. The time of deparaffination using scraper (which is still carried out in

these wells) decreased to 0.5 to 1 hours for Alg-556, and 10 to 20 minutes for Do-55, respectively. The frequency of mechanical cleaning is also decreased. This shows that the need for mechanical cleaning can be significantly reduced and faster, due to the significantly reduced precipitation.

The method of the invention can be suitable, in addition to the inhibition/removal of asphaltene-paraffin-vax precipitates, for the inhibition and removal of carbonate-sulphate precipitates, as well as for the inhibition of corrosion, by using appropriately selected microorganisms or mixtures thereof, which produce organic acids and/or other tenside intermediers.

Claims

1. Method for the removal of asphaltene-paraffin-vax precipitates and prevention of formation thereof on surfaces in contact with crude oil, comprising

a) adding tensides, materials for increasing viscosity, and microorganisms capable of breaking down crude oil components or derivatives and producing at least one type of tenside, to the surface, optionally together with additives required for the reproduction of said microorganisms;

b) providing an appropriate temperature for the microorganisms after the addition of the materials in step a);

c) allowing the microorganisms to reproduce and act for a predetermined period of time on the surface;

d) checking the results of the treatment; and

e) optionally repeating steps (a) to (d) at least once more, preferably at least three more times.

2. The method according to claim 1, wherein in step d) the results of the treatment are checked by confirming the presence of a film on the surface in contact with the crude oil which provides for the living conditions of the said microorganisms and contains the said microorganisms, and optionally steps a) to d) are repeated by changing the parameters, preferably by changing the amount of the tenside or material capable increasing viscosity, or by varying the reproduction time of microorganisms.

3. The method according to claim 1 or 2, wherein the said precipitates are removed from or prevented in the inner surfaces of tubings of oil-wells, flow lines thereof or in oil pipelines.

4. The method according to claims 1 to 3, wherein the said microorganisms and additives are added to the surface at the same time, in the form of an aqueous suspension.

5. The method according claim 4, wherein the suspension of microorganisms contains 10^6 to 10^{12} CFU/liter, preferably 10^7 to 10^{11} CFU/liter, more preferably 10^8 to 10^9 CFU/liter.

6. The method according to claim 4 or 5, wherein the volume of the suspension is 100 to 1000 liter/100 m pipe-length, preferably 300 to 800 liter/100 m pipe-length, more preferably 500 to 600 liter/100 m pipe-length.

7. The method according to claim 6, wherein the microorganisms are allowed to reproduce and act for 1 to 15 days, preferably for 6 to 8 days, while the pipes are kept closed.

8. The method according to claims 3 to 7, performed in a production oil-well, and the temperature in the well is determined by the geological conditions.

9. The method according to claims 3 to 8, wherein the results of the treatment are checked by pilot test and by mechanical cleaning test and/or by evaluating the physico-chemical properties, preferably the decrease of viscosity of an oil sample and/or evaluating the drop-size of the asphaltene-paraffin-vax precipitates in an oil-sample by microscopy.

10. The method according to claims 1 to 9, wherein the surfactant is selected from the group consisting of polyoxyethylene ethers and esters, and mixtures thereof, preferably Tween 80.

11. The method according to claims 1 to 10, wherein the material capable increasing viscosity is xanthan.

12. The method according to claims 1 to 11, wherein the asphaltene-paraffin-vax precipitates are removed from the surface in advance by mechanical means.

13. Use of a microorganism capable of breaking down crude oil components or derivatives and producing at least one type of tenside for the removal and prevention of asphaltene-paraffin-vax precipitates by way of forming a film carrying bacteria on surfaces in contact with crude oil.

14. The use according to claim 13 wherein the microorganism is a strain belonging to the *Bacillus subtilis* species, the *Bacillus cereus* species, the *Pseudomonas* genus or the *Xanthomonas* genus, and preferably facultative anaerobic.

15. The use according to claim 13 or 14 wherein the microorganism is selected form the group consisting strains NCAIM (P) B 1304, NCAIM (P) B 1305, NCAIM (P) B 1306, NCAIM (P) B 1307 and NCAIM (P) B 1308 deposited on April 17, 2002 at NCAIM, or any strain derived therefrom, and preferably is a strain that is genetically modified, more preferably modified by the insertion of a DNA fragment with a known sequence as a marker.

16. Kit for the removal or prevention of asphaltene-paraffin-vax precipitates on surfaces in contact with crude oil in pipelines, comprising a microorganism useful in the method of claim 1, further comprising instructions to carry out the method of any of claims 1 to 12.

17. The kit according to claim 16 comprising one or more of the microorganisms defined in any of claims 13 to 15 and additives necessary for the reproduction thereof.

18. The kit according to claim 16 or 17 further comprising a surfactant and/or a material for increasing viscosity.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/HU 03/00079

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 E21B37/06 E21B37/08 C12P1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 E21B C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
TULSA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PARTIDAS C J ET AL: "MICROBES AID HEAVY OIL RECOVERY IN VENEZUELA" OIL AND GAS JOURNAL, PENNWELL PUBLISHING CO. TULSA, US, vol. 96, no. 24, 15 June 1998 (1998-06-15), pages 62-64, XP000792944 ISSN: 0030-1388 the whole document	1-18
A	-& : BEN-BAC TECHNICAL DATA, 'Online! XP002272056 Retrieved from the Internet: <URL:www.micro-bac.com/pbstech.html> 'retrieved on 2004-02-26!	
A	-& PARA-BAC/S TECHNICAL DATA, 'Online! XP002272057 Retrieved from the Internet: <URL:www.micro-bac.com/bbtech.html> 'retrieved on 2004-02-26!	
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 March 2004	15/03/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Smalt, R

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/HU 03/00079

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
	the whole document	
X	LAZAR, I ET AL.: "The use of naturally occuring selectively isolated becteria for inhibiting paraffin deposition." JOURNAL OF PETROLEUM SCIENCE AND ENGENEERING, vol. 22, 1999, pages 161-9, XP002272058 the whole document	1-18
X	BLENKINSOPP, S.A. ET AL: "Parafin removal downhole" GAS, OIL, AND ENVIRONMENTAL BIOTECHNOLOGY, 1992, pages 419-25, XP008028040 the whole document	1-18
X	SCHNEIDER, D.R.: "Parafin control by the microbial product, Para-Bac" GAS, OIL, COAL AND ENVIRONMENTAL BIOTECHNOLOGY, 1991, pages 475-84, XP008028041 the whole document	1-18
A	SALLEE R T ET AL: "Biodegradation of used motor oil by indigenous soil microorganisms" BIOSIS, XP002243589 abstract	
A	ROJAS J O ET AL: "FLUIDIFICACION DE PETROLEO PESADO POR CULTIVOS BACTERIANOS HEAVY PETROLEUM FLUIDIZATION BY BACTERIAL CULTURES" ACTA CIENTIFICA VENEZOLANA, ASOCIACION VENEZOLANA PARA EL AVANCE DE LA CIENCIA, CARACAS, VE, vol. 47, no. 3, 1996, pages 154-159, XP008018052 ISSN: 0001-5504 abstract page 158, right-hand column, paragraph 3	
A	STABNIKOVA E V ET AL: "THE CHOICE OF ACTIVE HYDROCARBON-DESTRUCTING MICROORGANISM FOR CLEAN-UP OF OIL-POLLUTED SOILS" PRIKLADNAA BIOHIMIA I MIKROBIOLOGIA - APPLIED BIOCHEMISTRY AND MICROBIOLOGY, MOSCOW, RU, vol. 31, no. 5, 1995, pages 534-539, XP008018054 ISSN: 0555-1099 abstract	

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INTERNATIONAL SEARCH REPORT

International application No
PCT/HU 03/00079

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 161 511 A (UNIV ILLINOIS) 21 November 1985 (1985-11-21) the whole document	

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/HU 03/00079

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0161511 A	21-11-1985	DE 3580697 D1	10-01-1991
		EP 0161511 A2	21-11-1985
		US 5013654 A	07-05-1991

0-1	**Form - PCT/RO/134 (EASY) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)**	
0-1-1	Prepared using	PCT-EASY Version 2.92 (updated 01.07.2003)
0-2	**International Application No.**	PCT/HU03/00079
0-3	**Applicant's or agent's file reference**	99593-2967

1	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
1-1	page	8
1-2	line	1
1-3	**Identification of Deposit**	
1-3-1	Name of depositary institution	National Collection of Agricultural and Industrial Microorganisms (NCAIM)
1-3-2	Address of depositary institution	Department of Microbiology and Biotechnology University of Horticulture and the Food Industry, Somlói út 14-16, 1118 Budapest, Hungary
1-3-3	Date of deposit	17 April 2002 (17.04.2002)
1-3-4	Accession Number	NCAIM (P) B 001304
1-4	**Additional Indications**	NONE
1-5	**Designated States for Which Indications are Made**	all designated States
1-6	**Separate Furnishing of Indications**	NONE
	These indications will be submitted to the International Bureau later	
2	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
2-1	page	8
2-2	line	1

2-3	**Identification of Deposit**	
2-3-1	Name of depositary institution	National Collection of Agricultural and Industrial Microorganisms (NCAIM)
2-3-2	Address of depositary institution	Department of Microbiology and Biotechnology University of Horticulture and the Food Industry, Somlói út 14-16, 1118 Budapest, Hungary
2-3-3	Date of deposit	17 April 2002 (17.04.2002)
2-3-4	Accession Number	NCAIM (P) B 001305
2-4	***Additional Indications***	NONE
2-5	**Designated States for Which Indications are Made**	all designated States
2-6	**Separate Furnishing of Indications** These indications will be submitted to the International Bureau later	NONE
3	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
3-1	page	8
3-2	line	1
3-3	**Identification of Deposit**	
3-3-1	Name of depositary institution	National Collection of Agricultural and Industrial Microorganisms (NCAIM)
3-3-2	Address of depositary institution	Department of Microbiology and Biotechnology University of Horticulture and the Food Industry, Somlói út 14-16, 1118 Budapest, Hungary
3-3-3	Date of deposit	17 April 2002 (17.04.2002)
3-3-4	Accession Number	NCAIM (P) B 001306
3-4	**Additional Indications**	NONE
3-5	***Designated States for Which Indications are Made***	all designated States
3-6	**Separate Furnishing of Indications** These indications will be submitted to the International Bureau later	NONE
4	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
4-1	page	8
4-2	line	1

4-3	**Identification of Deposit**	
4-3-1	Name of depositary institution	National Collection of Agricultural and Industrial Microorganisms (NCAIM)
4-3-2	Address of depositary institution	Department of Microbiology and Biotechnology University of Horticulture and the Food Industry, Somlói út 14-16, 1118 Budapest, Hungary
4-3-3	Date of deposit	17 April 2002 (17.04.2002)
4-3-4	Accession Number	NCAIM (P) B 001307
4-4	**Additional Indications**	NONE
4-5	**Designated States for Which Indications are Made**	all designated States
4-6	**Separate Furnishing of Indications** These indications will be submitted to the International Bureau later	NONE
5	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
5-1	page	8
5-2	line	1
5-3	**Identification of Deposit**	
5-3-1	Name of depositary institution	National Collection of Agricultural and Industrial Microorganisms (NCAIM)
5-3-2	Address of depositary institution	Department of Microbiology and Biotechnology University of Horticulture and the Food Industry, Somlói út 14-16, 1118 Budapest, Hungary
5-3-3	Date of deposit	17 April 2002 (17.04.2002)
5-3-4	Accession Number	NCAIM (P) B 001308
5-4	**Additional Indications**	NONE
5-5	**Designated States for Which Indications are Made**	all designated States
5-6	**Separate Furnishing of Indications** These indications will be submitted to the International Bureau later	NONE

FOR RECEIVING OFFICE USE ONLY

0-4	**This form was received with the international application:** (yes or no)	YES
0-4-1	Authorized officer	**Dr. Susanna PÁCZAY** authorized officer

0-5	**This form was received by the international Bureau on:**	
0-5-1	Authorized officer	

[redacted]CT/HU2003/000079

BUDAPEST TREATY ON THE INTERNA[redacted]
RECOGNITION OF THE DEPOSIT OF MICRORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

TO: **MOL Hungarian Oil and Gas PLC**
H-1117 Budapest
18, Október 23 Street

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITORY AUTHORITY
identifled at the bottom of this page

NAME AND ADDRESS
OF DEPOSITOR

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the Depositor: **mol-2**

Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
NCAIM (P) B 001304

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorgnnism identified under I. above was accompanied by:

☐ a scientific description

☐ a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depository Authority accepts the microorganism identified under I above, which was received by it on **April 17, 2002** (date of the original deposit)[1]

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I. above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: **National Collection of Agricultural and Industrial Microorganisms**

Address: **Budapest Somlói út 14-16.**
1118
HUNGARY

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s)

Date: **June 13, 2002**

( )[1] Where Rule 6.l (d) applies,such date is the date on which the status of internationnl depositary authority was acquired.

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PCT/HU2003/000079

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICRORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

TO: **MOL Hungarian Oil and Gas PLC**
H-1117 Budapest
18, Október 23 Street

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITORY AUTHORITY
identified at the bottom of this page

NAME AND ADDRESS
OF DEPOSITOR

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the Depositor: **mol-32**

Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
NCAIM (P) B 001305

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorgnnism identified under I. above was accompanied by:

☐ a scientific description

☐ a proposed taxonomic designation

(Mark with a cross where ăpplicable)

III. RECEIPT AND ACCEPTANCE

This International Depository Authority accepts the microorganism identified under I above, which was received by it on **April 17, 2002** (date of the original deposit)[1]

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I. above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: **National Collection of Agricultural and Industrial Microorganisms**

Address: **Budapest Somlói út 14-16.**
1118
HUNGARY

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s)

Date: **June 13, 2002**

( )[1] Where Rule 6.1 (d) applies,such date is the date on which the status of internationnl depositary authority was acquired.

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[redacted]APEST TREATY ON THE INTERNATIO[redacted] PCT/HU2003/000079
RECOGNITION OF THE DEPOSIT OF MICRORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

TO: **MOL Hungarian Oil and Gas PLC**
H-1117 Budapest
18, Október 23 Street

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITORY AUTHORITY
identified at the bottom of this page

NAME AND ADDRESS
OF DEPOSITOR

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the Depositor: **mol-51**	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: **NCAIM (P) B 001306**

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorgnnism identified under I. above was accompanied by:

☐ a scientific description

☐ a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depository Authority accepts the microorganism identified under I above, which was received by it on **April 17, 2002** (date of the original deposit)[1]

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I. above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: **National Collection of Agricultural and Industrial Microorganisms**

Address: **Budapest Somlói út 14-16.**
1118
HUNGARY

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s)

Date: **June 13, 2002**

( )[1] Where Rule 6.1 (d) applies,such date is the date on which the status of international depositary authority was acquired.

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PCT/HU2003/000079

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICRORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

TO: **MOL Hungarian Oil and Gas PLC**
H-1117 Budapest
18, Október 23 Street

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITORY AUTHORITY
identified at the bottom of this page

NAME AND ADDRESS
OF DEPOSITOR

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the Depositor: **mol-66**

Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
NCAIM (P) B 001307

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorgnnism identified under I. above was accompanied by:

☐ a scientific description

☐ a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depository Authority accepts the microorganism identified under I above, which was received by it on **April 17, 2002** (date of the original deposit)[1]

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I. above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: **National Collection of Agricultural and Industrial Microorganisms**

Address: **Budapest Somlói út 14-16.**
1118
HUNGARY

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s)

Date: **June 13, 2002**

( )[1] Where Rule 6.1 (d) applies,such date is the date on which the status of internationnl depositary authority was acquired

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[redacted]PEST TREATY ON THE INTERNATIO[redacted] PCT/HU2003/000079
RECOGNITION OF THE DEPOSIT OF MICRORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

TO: **MOL Hungarian Oil and Gas PLC**
H-1117 Budapest
18, Október 23 Street

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITORY AUTHORITY
identifled at the bottom of this page

NAME AND ADDRESS
OF DEPOSITOR

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the Depositor: **mol-107**

Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
NCAIM (P) B 001308

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorgnnism identified under I. above was accompanied by:

☐ a scientific description

☐ a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depository Authority accepts the microorganism identified under I above, which was received by it on **April 17, 2002** (date of the original deposit)[1]

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I. above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: **National Collection of Agricultural and Industrial Microorganisms**

Address: **Budapest Somlói út 14-16.**
1118
HUNGARY

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s)

Date: **June 13, 2002**

( )[1] Where Rule 6.1 (d) applies,such date is the date on which the status of internationnl depositary authority was acquired

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